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DENERVATED SKELETAL MUSCLE AND ON
THE ACETYLCHOLINE AND PHY-
SOSTIGMINE ACTIONS ON
SKELETAL MUSCLE

A PART OF A DISSERTATION SUBMITTED
TO THE FACULTY OF THE DIVISION OF THE
BIOLOGICAL SCIENCES IN CANDIDACY FOR
THE DEGREE OF DOCTOR OF PHILOSOPHY

DEPARTMENT OF PHYSIOLOGY

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QUININE: EFFECTS ON NORMAL AND DENERVATED SKELETAL MUSCLE AND ON THE ACETYLCHOLINE AND PHYSOSTIGMINE ACTIONS ON SKELETAL MUSCLE¹

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The statement made by Sollmann (1) that quinine, quinidine, cinchonine, and cinchonidine produce muscular depression with dilute solutions and toxic rigor with more concentrated solutions is apparently based mainly upon work done with isolated nerve-muscle preparations of the frog. Piccinini (2, 3) investigated the effects of quinine on the frog nerve-muscle preparation and also in the intact frog. He reported that small doses increased the muscle response. Smith and Fantus (4) working with frogs reported that the tetanus response to nerve faradization was changed to a single twitch response after quinine, although the muscle still responded fully to direct stimulation. Vondracek (5) ran ergographic and dynamometer experiments on a group of soldiers and concluded that quinine increased the muscle work not so much through direct muscle action but through action on the central nervous mechanism. The recent report of Kolb, Harvey and Whitehall (6) in which quinine sulphate abolished the myotonus of myotonia atrophica but had no effect on muscular strength indicates that in the myotonic patient no depression

¹ Since the preparation of this article, an abstract in the proceedings of the XVI International Physiological Congresses on *The Actions of Quinine on Skeletal Muscle* by A. M. Harvey has become available. (Kongressbericht II des XVI Internationalen Physiologen-Kongresses, page 183, 1938, Zurich). In general, the similar type of experiments which are summarized by Harvey agree with those reported here.

is obtained such as was reported by Brody and Sollmann (7) for isolated frog nerve-muscle preparations, nor was there any increase in muscle work such as was reported by Vondracek in normal man. An older report on the action of quinine on skeletal muscle is that of Santesson (8). He reported an increased extensibility of frog skeletal muscle after quinine in response to single stimuli. However, in fatigue experiments the quinine treated muscle fatigued more quickly and the total amount of work done was less than in the untreated muscle. Tetanus experiments indicated a decreased response after the use of quinine. These results were confirmed on the skeletal muscle of the rabbit. Secher (9) reported depression of muscle contraction by quinine and related alkaloids.

A few other reports complete the available literature on the effects of quinine or its related alkaloids on skeletal muscle. Weiss (10) found that quinine and quinidine prevented the fibrillation of skeletal muscle after eserine, in the dog. Buchbinder (11) determined that quinidine had an inhibitory influence on the fibrillation of the dog's tongue following hypoglossotomy.

The lack of a detailed report on the effects of quinine on the mammalian skeletal neuro-muscular system led to the present study. The recent consistently successful alleviation of the clinical symptoms of myotonia congenita by the oral use of quinine (12-16) suggested a further problem concerning the possible mechanism by which quinine brings about its effective relief of the symptoms of myotonia. In addition to this the clinical usefulness of quinine as a diagnostic agent for myasthenia gravis has been suggested (16, 6). A physostigmine- or prostigmine-treated myasthenia patient, relieved of his symptoms, is readily precipitated to the pre-treatment level by the use of quinine. On the other hand, the patient who has been relieved of his myotonus symptoms by quinine is returned to the pre-quinine level by the use of prostigmine or physostigmine. Here is a clinical antagonism which when studied in the experimental animal might yield some information as to the nature of the action of these drugs on skeletal muscle. Finally the detailed study of this antagonism and its influence on the acetylcholine picture

should provide further evidence as to the nature of the transmission of the nerve impulse to skeletal muscle.

METHOD

Dogs were used in all experiments. Pentobarbital (Nembutal) anesthesia was employed, usually 45 mgm. per kilogram, intraperitoneally. The tendon of the anterior tibialis muscle was freed and sectioned near its point of attachment. Subsequently, the contractions of the muscle were recorded on a kymograph record by attaching the tendon to a steel spring myograph which in turn activated a light lever pulling against a strong rubber band. The leg was firmly fixed to a rigid support by means of rods drilled into the tibia. The sciatic nerve was sectioned high in the thigh. The peroneal nerve was isolated and was used for subsequent indirect stimulation of the muscle. Shielded platinum electrodes were employed. (Denervated muscles were stimulated by means of steel needles thrust into the muscle substance). Stimulation was provided by condenser discharges (capacity of 0.01 to 0.2 microfarad). The variable time interval was controlled by a neon tube circuit. A stimulator as described by Bernstein (17) was used to deliver pairs of stimuli separated by a very short interval of time. In most experiments the basic rate of stimulation of once every eight to ten seconds was maintained throughout. However, in experiments where curare was used, this interval was raised to one every fifteen seconds. A tracheal cannula was routinely inserted since artificial respiration was generally required in the experiments where physostigmine, prostigmine, or curare were used. Injections were made through the femoral vein of the opposite limb or in some cases the jugular vein was used. Atropine sulphate, 1 or 2 mgm. per kilogram, was administered in experiments where physostigmine, prostigmine, or acetylcholine were used. The atropine served to diminish the vascular and cardiac effects and caused no effect on the control muscle response nor did it change the response of the muscle to quinine. Acetylcholine bromide, physostigmine salicylate, and quinine dihydrochloride were the salts used. Curare

was made from crude material obtained from South American natives. A simple maceration was made and diluted as required.

RESULTS

Quinine

The injection of quinine dihydrochloride in doses from 5 to 40 mgm. per kilogram body weight, increased the magnitude of the responses of the anterior tibialis muscle to single condenser discharges applied to the peroneal nerve. This result was obtained

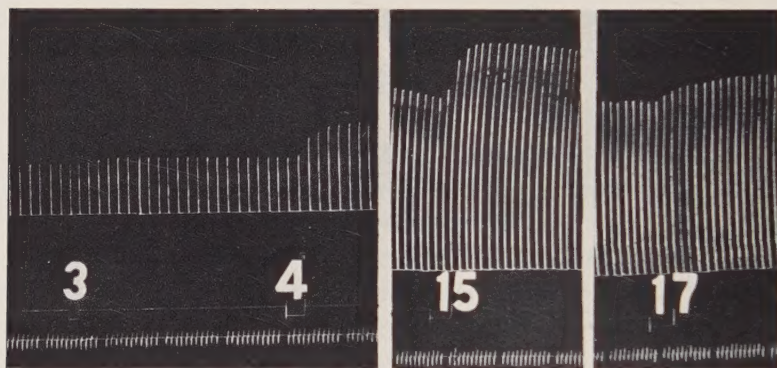


FIG. 1. POTENTIATION OF MUSCLE RESPONSE BY QUININE

In this and in the following records, unless otherwise stated, upper record, muscle contraction response; middle record, injection marker; lower record, time in five second intervals; nembutal, 45. mgm. per kilogram, intra-peritoneally, at least two hours previously; atropine, 1 mgm. per kilogram. No atropine in figure 1.

3, five mgm. per kilogram quinine; 4, twenty mgm. per kilogram quinine; 15, forty mgm. per kilogram quinine in another animal; 17, forty mgm. per kilogram quinine (33 minutes after 15).

with the rate of stimulation varied from one per ten seconds to two per second. The potentiation was of small magnitude after the smallest dose and increased with the increased dosage (fig. 1). The potentiating effect generally reached its maximum within one or two minutes after the intravenous administration of the drug. After the use of one mgm. per kilogram no potentiation was noted.

With very large amounts of quinine, that is, up to 160 mgm. per kilogram in divided doses over a period of time, the effect on

the contraction curve resulting from *tetanic* stimulation was one of depression. The tetanus plateau, instead of remaining smooth

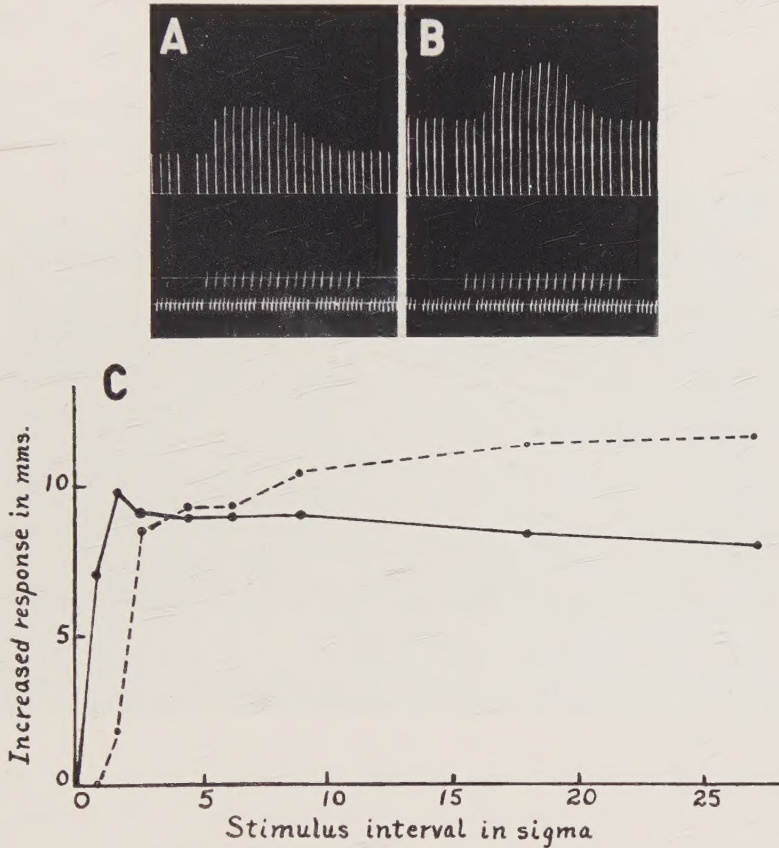


FIG. 2. INFLUENCE OF QUININE ON SUMMATION CURVE

A. Signal marker indicates application of stimulation to peroneal nerve. First and second stimuli are single. The successive marked stimuli are double stimuli applied 0.9, 1.8, 2.7, 4.5, 6.3, 9.0, 18, 27, 36, 45, 63, 81, and 90 milliseconds apart, respectively. The last two marked stimuli are the original first and second repeated.

B. A repetition of A after 25 mgm. per kilogram quinine.

C. Response of muscle plotted against intervals between paired stimuli, taken from A and B. Solid curve, normal; broken curve, after quinine; zero equals the normal twitch height.

as in the control, may become ragged. The maximum height was lowered and the ability to sustain tetanus was diminished.

Another finding subsequent to the injection of quinine was a prolongation of the least effective interval for the summation of two successive stimuli. The curve and records in figure 2 show this effect.

Physostigmine

The normal response of a muscle, stimulated at the rate of once every ten seconds, to the intravenous injection of physostigmine is a gradual potentiation of the muscle twitches (18). If quinine is administered before physostigmine the potentiation normally produced by the physostigmine may be completely blocked

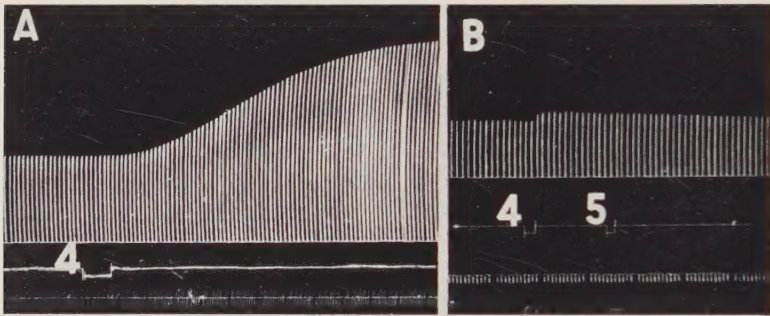


FIG. 3. BLOCK OF PHYSOSTIGMINE POTENTIATION OF MUSCLE RESPONSE BY QUININE

Dog A: 4, 0.1 mgm. per kilogram physostigmine; dog B: 4, twenty mgm. per kilogram quinine (20 mgm. per kilogram 15 minutes before); 5, 0.5 mgm. per kilogram physostigmine.

(fig. 3). This effect seems rather remarkable in view of the potentiation which is produced by quinine itself. If quinine is given after physostigmine, the physostigmine (potentiated) level of contraction is reduced. The final level of contraction height is very close to that which would obtain in the presence of quinine alone: intermediate between the control or pre-drug level and the maximum physostigmine-potentiated level (fig. 4). This antagonism can be shifted in either direction depending upon the proper dosage proportion.

Quinidine and prostigmine have been substituted for one or the other, quinine or physostigmine, respectively, in several experiments and yielded the same type of result.

Acetylcholine

Acetylcholine, 0.5 to 5 mgm. per kilogram, produces very little effect on normal muscle twitches (19). There is no change in this result if acetylcholine is given after quinine. The well-known acetylcholine response after physostigmine can be easily obtained (fig. 5). If quinine be given soon after physostigmine this picture may be greatly modified. By appropriate proportion

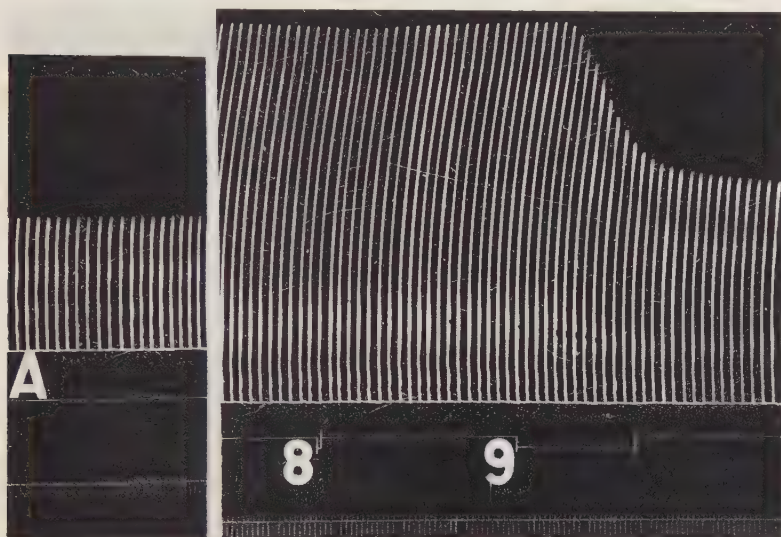


FIG. 4. RELEASE OF PHYSOSTIGMINE POTENTIATION BY QUININE

A, normal height before injection of drugs; 8, 0.1 mgm. per kilogram physostigmine (0.3 mgm. per kilogram 20 minutes before). 9, 40 mgm. per kilogram quinine.

of dosage the acetylcholine post-physostigmine effect can be partially or completely prevented (fig. 5). The same effect is produced if quinine is administered first and then followed by physostigmine.

Curare

Physostigmine is recognized as a decurarizing substance (19). In view of the antagonisms already shown to exist between quinine and physostigmine, it was deemed advisable to determine

the effects of these two drugs upon the curarized preparation. In no experiment did quinine produce any decurarization. In fact, it caused an additional depression of twitches in those animals which were only partially curarized. Quinine, then, augments curarization while physostigmine decurarizes.

Acetylcholine produces in the control curarized preparation a transitory partial decurarization. In the curarized preparation

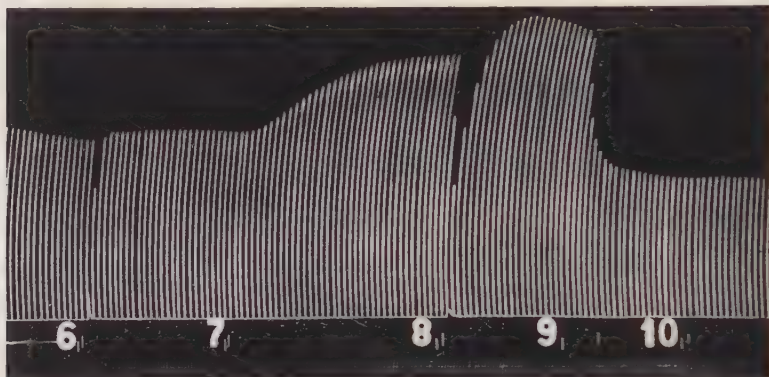


FIG. 5. RELEASE OF PHYSOSTIGMINE POTENTIATION AND BLOCK OF ACETYLCHOLINE BY QUININE

6, one mgm. per kilogram acetylcholine (0.1 mgm. per kilogram physostigmine 30 minutes before); 7, 0.1 mgm. per kilogram physostigmine; 8, one mgm. per kilogram acetylcholine; 9, forty mgm. per kilogram quinine; 10, one mgm. per kilogram acetylcholine.

to which quinine has been given, acetylcholine may become ineffective as a decurarizing substance.

Denervated muscle

The denervated muscle is very sensitive to acetylcholine (20, 21). Physostigmine does not potentiate the twitches of the denervated muscle as it does those of the innervated muscle (21). Two components of the acetylcholine response of the denervated muscle have been described (21). The contraction phase occurs very soon, and is followed by a contracture phase. Refractoriness is generally present to some extent during the contracture phase. These facts were amply confirmed in this work. In six animals, with the peroneal nerve sectioned from 9

to 12 days previously, the following observations were made after the injection of quinine:

1. Very slow muscle twitch potentiation (fig. 6).
2. Decrease in the height of tetanus.
3. Very slight raggedness of the tetanus plateau.

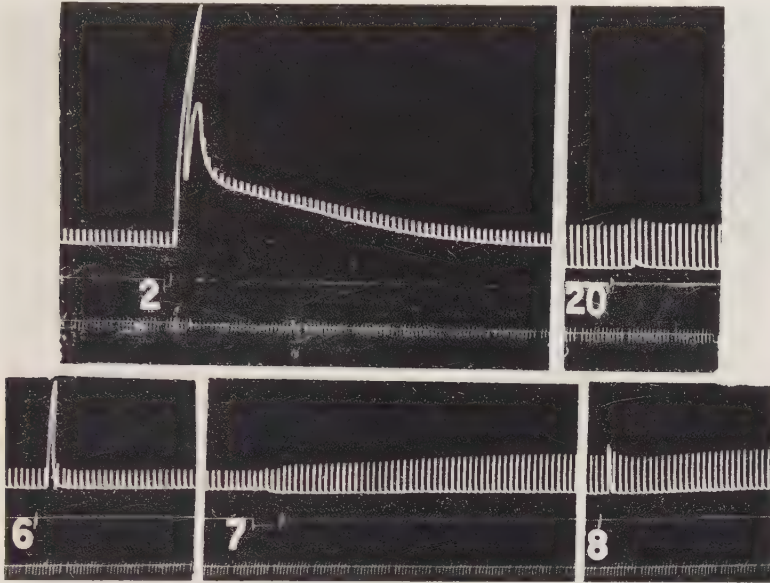


FIG. 6. MUSCLE RESPONSE POTENTIATION AFTER QUININE IN A MUSCLE 11 DAYS AFTER NERVE SECTION

Acetylcholine rendered ineffective.

Dog A: 2, 0.1 mgm. per kilogram acetylcholine before quinine; 20, 0.1 mgm. per kilogram acetylcholine—100 mgm. per kilogram quinine during the hour between 2 and 20.

Dog B: 6, 0.1 mgm. per kilogram acetylcholine; 7, forty mgm. per kilogram quinine; 8, 0.1 mgm. per kilogram acetylcholine.

The administration of quinine made the subsequent acetylcholine injections less effective than the sensitive response before quinine (fig. 6). By appropriate dosage the acetylcholine may be rendered completely ineffective.

DISCUSSION

The results obtained in this series of experiments do not agree with the general statement that quinine produces muscular de-

pression. In both the innervated and denervated muscle, quinine produced potentiation of the muscle twitches. However, depression of tetanic responses was noted especially in larger doses in the innervated preparation. The maximum potentiation in the innervated muscle is reached within about two minutes after injection of quinine while in the denervated muscle the maximum is reached only after six to ten minutes. Since it does take place in the denervated preparation, potentiation is probably a direct muscle effect; at least it does not depend upon innervation for its production.

Three facts taken from these experiments support the idea that quinine prevents acetylcholine from acting on the nerve-muscle preparation and is, therefore, an acetylcholine inhibitor:

1. The normal decurarization action of acetylcholine may be prevented by quinine injection.
2. The highly sensitive response of the denervated muscle to acetylcholine is prevented by the use of quinine.
3. The physostigmine protection of acetylcholine in the normal muscle is prevented by the administration of quinine.

Quinine, then, has an influence on the action of acetylcholine on voluntary muscle: it prevents acetylcholine from producing its normal effect. The site of the reaction may be beyond the point of innervation, since it is readily demonstrated in the denervated muscle. Possibly these two mechanisms are involved:

1. A block in the acetylcholine reaction with muscle, or
2. Increased destruction of acetylcholine.

It is difficult to see how a substance which prevents acetylcholine potentiation will itself potentiate at the same time. This is possible, of course, if quinine substitutes itself for acetylcholine in a particular reaction. It would then act as an effective block for acetylcholine and might itself be a substance which, when involved in the reaction, would potentiate. Another logical explanation is to assume that quinine has two separate actions on skeletal muscle, one influencing the magnitude of individual responses and the other antagonizing acetylcholine effects. An anti-acetylcholine effect of quinine has been demonstrated by Stavraký (22) on the output of saliva. In his experiments

quinine abolished the ability of acetylcholine to increase the flow of saliva. Starr (23) has shown that quinidine may abolish or diminish the ability of acetylcholine to decrease the heart rate. This result was demonstrated in the isolated cat and rabbit hearts and also in the intact cat.

Realizing that caution should be exercised in carrying over conclusions based upon experiments in normal animals to pathological conditions, it is nevertheless of value to note several interesting observations made on normal animals which may aid in our understanding of myotonia. It has become increasingly evident that myotonia is a peripheral disorder, especially in the light of experiments on patients with spinal anesthesia (24) where the myotonic reaction still persists and is readily diminished by the use of quinine. Lanari (25) has demonstrated a sensitivity of the muscles of the myotonic patient to acetylcholine. Kennedy and Wolf (24) theorize that quinine is effective in myotonia through inhibition of acetylcholine, acting at the myoneural junction. The experimental observations that quinine blocks acetylcholine action, especially in the hypersensitive muscle (in these experiments, the denervated muscle) is of special significance in relation to the use of quinine in myotonia. Quinine given to the myotonic patient may remove the myotonus by preventing acetylcholine from stimulating the myotonic muscle, which is abnormally sensitive to acetylcholine.

Quinine causes the return of myasthenia symptoms in a prostigmine-treated patient (6). This would appear to be associated with the muscle potentiation effect of prostigmine and with the release of this effect by quinine. A patient whose muscles are practically powerless is restored, temporarily, to power by the use of the potentiating prostigmine while the former powerless condition is restored with the release or block of this potentiating condition by quinine. Similarly, the reappearance of myotonic symptoms in the quinine-treated patient by the use of prostigmine is probably due to the acetylcholine protecting action of the prostigmine. This treatment permits a sufficient concentration of acetylcholine to produce myotonus in the sensitive muscle.

The results which have been obtained in this series of experi-

ments are explained in relation to the theory, originally advanced by Dale and Feldberg (26) and subsequently elaborated and supported (27, 28, 29), that acetylcholine is a chemical mediator in the transmission of the nerve impulse to voluntary muscle. They are, indirectly, therefore, further evidence in favor of this theory.

SUMMARY

Quinine produces the following effects on the *in vivo* nerve-muscle preparation of the dog:

1. Potentiation of muscle twitches.
2. Depression of muscle tetanus, especially in larger doses.
3. Prolongation of the least effective interval for the summation of two successive stimuli.
4. Block of the normal physostigmine potentiation.
5. Block of the post-physostigmine acetylcholine muscle effects.
6. Additional depression of muscle twitches in a partially curarized animal.
7. Block of the acetylcholine decurarization.
8. Potentiation of the denervated muscle twitch.
9. Block of the acetylcholine effects on the denervated muscle.

The relation and significance of these findings to the clinical use of quinine and physostigmine in myotonia and myasthenia are discussed.

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